

## APPENDIX A

### CLAIMS (clean)

#### (arranged in logical rather than numerical order)

1. (*currently amended*): A cultured eukaryotic cell transformed with a nucleic acid expression construct which construct comprises:

- (a) a nucleotide sequence that encodes xylose isomerase the amino acid sequence of which is at least 95% identical with SEQ ID NO:1, and
- (b) operatively linked to the nucleotide sequence of (a), a promoter that drives active expression of the xylose isomerase coding sequence in the transformed cell,

wherein, said expression construct is expressible in said cell and expression thereof confers on the cell the ability to directly isomerize xylose to xylulose.

#### **21 – 23 CANCELLED**

24. (*currently amended*) A transformed eukaryotic according to claim 1, wherein the nucleotide sequence encodes a xylose isomerase the amino acid sequence of which is SEQ ID NO:1.

25. (*new*) The transformed eukaryotic cell according to claim 1, wherein the amino acid sequence of the encoded xylose isomerase is identical to that of a xylose isomerase that naturally occurs in a eukaryotic organism.

2. (*previously presented*): A transformed host cell according to claim 1, wherein the cell is a yeast cell.

18. (*previously presented*) The yeast cell of claim 2 that is a member of a genus selected from the group consisting of *Saccharomyces*, *Kluyveromyces*, *Candida*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kloeckera*, *Schwanniomyces*, and *Yarrowia*.

3. (*previously presented*) The yeast cell according to claim 18 that is a member of a species selected from the group consisting of *S. cerevisiae*, *S. bulderi*, *S. barnetti*, *S. exiguis*, *S. uvarum*, *S. diastaticus*, *K. lactis*, *K. marxianus*, and *K. fragilis*.

4. (*previously presented*): A transformed cell according to claim 1, wherein the cell is a filamentous fungus.

19. (*previously presented*) The filamentous fungus cell of claim 4 that is a member of a genus selected from the group consisting of *Aspergillus*, *Trichoderma*, *Humicola*, *Acremonium*, *Fusarium*, and *Penicillium*.

#### **5. Cancelled**

6. (*previously presented*) A transformed cell according to claim 1, wherein the promoter is insensitive to catabolite repression in the cell.

## APPENDIX A (cont.)

7. (*currently amended*) A cultured transformed eukaryotic cell that has been genetically modified to confer on the cell one or more of the following properties:

- (1) increased transport of xylose into the host cell;
- (2) increased xylulose kinase activity;
- (3) increased flux of the pentose phosphate pathway;
- (4) decreased sensitivity to catabolite repression;
- (5) increased tolerance to ethanol, osmolarity or organic acids; or
- (6) decreased production of by-products,

in comparison to a similar cell that has not undergone said genetic modification, and

which cell is transformed with a nucleic acid expression construct that comprises:

- (a) a nucleotide sequence that encodes xylose isomerase the amino acid sequence of which is at least 95% identical with SEQ ID NO:1, and
- (b) operatively linked to the nucleotide sequence of (a), a promoter that drives active expression of the xylose isomerase coding sequence in the transformed cell,

wherein, said expression construct is expressible in said cell and active expression thereof confers on the cell, as an additional property, the ability to directly isomerize xylose to xylulose.

26. (*new*) The transformed eukaryotic cell according to claim 7, wherein the nucleotide sequence encodes a xylose isomerase the amino acid sequence of which is SEQ ID NO:1.

27. (*new*) The transformed eukaryotic cell according to claim 7, wherein the amino acid sequence of the encoded xylose isomerase is identical to that of a xylose isomerase that naturally occurs in a eukaryotic organism.

8. (*currently amended*) A transformed cell according to claim 7, wherein the genetic modification that results in said properties (1) – (6) is

- (i) overexpression of an endogenous gene,
- (ii) expression of a heterologous gene, or
- (iii) a combination of (i) and (ii), and

wherein the heterologous gene being expressed or the endogenous gene being overexpressed is a gene encoding:

- (a) a hexose transporter;
- (b) a pentose transporter;
- (c) a xylulose kinase;
- (d) an enzyme from the pentose phosphate pathway,
- (e) a glycolytic enzyme, or
- (f) an ethanologenic enzyme.

9. (*currently amended*) A transformed cell according to claim 7, wherein the genetic modification that results in said properties (1) – (6) is one that causes inactivation of one of the following endogenous genes:

- (a) a gene encoding a hexose kinase
- (b) *Saccharomyces MIG1* gene;
- (c) *Saccharomyces MIG2* gene; or

## APPENDIX A (cont.)

(d) a gene homologous to (a), (b) or (c) and which hybridizes thereto.

10. (*currently amended*) A cultured yeast cell that: expresses one or more enzymes that confers on the cell the ability to produce a fermentation product selected from the group consisting of lactic acid, acetic acid, succinic acid, amino acids, 1,3-propanediol, ethylene, glycerol, a  $\beta$ -lactam antibiotic and a cephalosporin; and

which cell is transformed with a nucleic acid expression construct which construct comprises:

- (a) a nucleotide sequence that encodes xylose isomerase the amino acid sequence of which is at least 95% identical with SEQ ID NO:1, and
- (b) operatively linked to the nucleotide sequence of (a), a promoter that drives active expression of the xylose isomerase coding sequence in the transformed cell,

wherein, expression of the construct confers on the cell the ability to directly isomerize xylose to xylulose and improve production of said fermentation product.

28. (*new*) The transformed eukaryotic cell according to claim 10, wherein the amino acid sequence of the encoded xylose isomerase is identical to that of a xylose isomerase that naturally occurs in a eukaryotic organism.

11. (*proposed amended*) A transformed yeast cell according to claim 10 in which alcohol dehydrogenase activity is genetically decreased so as to reduce ethanol production by said cell.

12. (*previously presented*) A process for producing ethanol, comprising the steps of:

- (a) fermenting a medium containing a source of xylose with the transformed cell of claim 1, which cell ferments xylose to ethanol, and, optionally,
- (b) recovering he ethanol.

13. (*previously presented*) A process according to claim 12, wherein the medium also contains a source of glucose.

14. (*previously presented*) A process according to claim 12 wherein the production of ethanol occurs at a rate of at least 0.5 g ethanol per liter per hour.

15. (*previously presented*) A process according to claim 12, wherein the ethanol yield is at least 50%.

16. (*previously presented*) A process for producing, as a fermentation product, lactic acid, acetic acid, succinic acid, an amino acid, 1,3-propanediol, ethylene, glycerol, a  $\beta$ -lactam antibiotic or a cephalosporin, which process comprises the steps of:

- (a) fermenting a medium containing a source of xylose with the transformed cell of claim 10, which cell ferments xylose to yield the fermentation product, and, optionally,
- (b) recovering the fermentation product.

**APPENDIX A (cont.)**

17. *(previously presented)* A process according to claim 16, wherein the medium also contains a source of glucose.
20. *(previously presented)* The process of claim 16 wherein the cell further comprises a genetic modification that results in decreased alcohol dehydrogenase activity.